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Synthesis of Diketopiperazine Scaffolds with tailored *N*- and α-chains by Selective Modification of Customizable Units

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Supporting information for this article is available on the WWW under DOI: 10.1002/adsc.202000470. It includes PDF file with recipes for the preparation of substrates **3** and **6**, and products **8-14** and **15-29**, and reproductions of ^{1}H and ^{13}C NMR spectra of these new compounds.

Abstract. The selective manipulation of Hyp customizable units in DKP substrates allows the generation of a rigid scaffold with four tailor-made chains which are spatially-orientated. The key step is a domino radical scission-oxidation process which allows the generation of *N*-substituted DKPs.

The versatility of this methodology to produce scaffolds in high optical purity for material and drug discovery is described herein

Keywords: chemoselectivity, domino reactions, organic synthesis, peptides, sustainable chemistry

Introduction

The site-selective modification of peptides through scission of "customizable units" such as Hyp, Ser/Thr, or Glu, and further manipulation of the resulting chains, has allowed the introduction of unnatural amino acids with a diversity of functionalized chains.[1,2] These chains can present groups for molecular imaging, organocatalysis, or interaction with biological/pharmacological targets.[3]

The interaction with receptors is favored when a rigid scaffold orientates the functionalized chains in the appropriate directions. The diketopiperazine (DKP) scaffold (**Figure 1**) seemed particularly promising,[4] since it is readily formed from inexpensive materials, it can be functionalized with four different chains, and remarkably, the chains on C-α would be oriented in opposite directions to the *N*-substituents (structure **1**).[5,6] In this way, multiple interactions with different biological receptors or with different residues in a certain active center could be achieved. The cyclic dipeptides could also be attached to form dimers, oligomers or polymers (structure **2**), increasing the number of functional groups.[7,8] The formation of DKP-containing polymers has scarcely been explored, particularly the use of tetrasubstituted DKPs as polymer units as shown in structure **2**.

We have recently been interested in peptide-derived scaffolds that can carry drugs or medical probes through biological membranes, in particular bacterial membranes.[8,9] It is known that peptides with cationic residues strongly interact with bacterial membranes, which are negatively-charged.[10] Since eukaryotic membranes are almost neutral, their interaction with the cationic peptides is reduced. If the peptides combine cationic and hydrophobic residues in an appropriate ratio, promising antimicrobials can result, displaying good target selectivity.[10,11] Based on this information, it would be interesting to design DKP scaffolds (**1**, Figure 1) with a combination of cationic and hydrophobic chains, whose spatial orientation favored cell penetration.

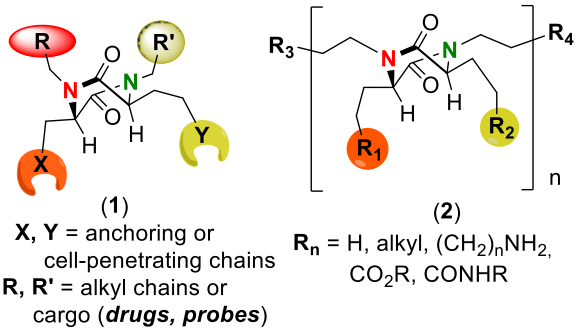
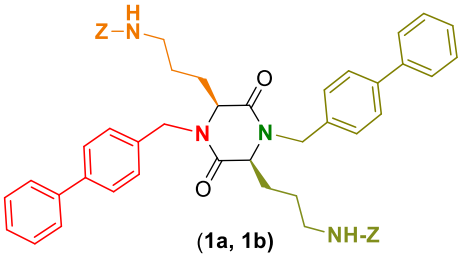


Figure 1. DKP scaffolds for biomedical applications.

Natural and synthetic DKPs are known to display antimicrobial[4,12] and quorum quenching activity,[4,13] but most are *N*-unsubstituted or *N*-monosubstituted (often *N*-methylated). Recently synthetic antimicrobial DKPs with two cationic and two hydrophobic residues were reported by Svenson et al (compounds **1a** and **1b**, Figure 2).[14] In this work,

only tetrasubstituted DKPs with bulky *N*-substituents such as the 4-phenyl benzyl group displayed a potent, broad-spectrum antibacterial activity. Interestingly, the most active compounds **1a** and **1b** would match the model scaffold **1**, with the two cationic substituents as cell-penetrating α-chains X and Y.



(**1a**, **1b**)

MIC	[**1a**] **Z** = H	[**1b**] **Z** = NHC(=NH)-NH_2
E. coli	32 μg/mL	32 μg/mL
P.aeruginosa	8 μg/mL	16 μg/mL
MRSA	4 μg/mL	2 μg/mL
S. epiderimidis	8 μg/mL	2 μg/mL

Figure 2. Reported DKP scaffolds with potent antimicrobial activity [Svenson et al, ref. 14].

The DKPs **1a/1b** presented two identical cationic and two identical hydrophobic units. In contrast, this communication will address the synthesis of DKP scaffolds with four different chains, which could be tailored to be cationic or hydrophobic residues.

The synthesis of such scaffolds is not simple. In spite of the ready formation of DKP rings in usual peptides,[4] and even *N*-methyl substituted cyclic dipeptides,[15] when other *N*-alkyl substituted DKPs are required, the synthesis is more complex.[16,17] The formation of tetrasubstituted DKPs often proceeds in low yields, with long reaction times or epimerization problems.[4,16-17] In the case of the DKPs **1a/1b**, a strong base and a very reactive electrophile (4-phenyl benzyl bromide) were used to introduce the bulky hydrophobic residues at both *N*-positions.[14a]

In another interesting work, Luthman et al reported that the cyclization of terminal *N*-substituted dipeptides to the desired tetrasubstituted DKPs failed. Therefore, an alternative route was developed, where a trisubstituted DKP was synthesized, using high temperatures under microwave irradiation, and then the last *N*-substituent was introduced using highly reactive electrophilic reagents (propargyl, allyl or benzyl bromides, an alkyl iodide).[16] These examples are representative of the difficulties found by different groups to produce tetrasubstituted DKPs.[17]

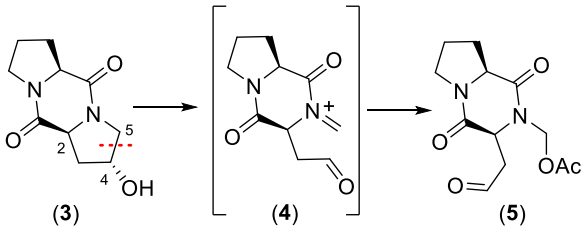
In addition, when non-natural residues are involved, the synthetic challenge increases. We report a method in which a single DKP substrate with two customizable Hyp units can afford a library of DKP scaffolds with four different chains for biomedical SAR studies, including bulky *N*-alkyl substituents. Other advantages are the mild reaction conditions, and the preservation of the stereochemistry of the initial Hyp units. [18]

Finally, we will comment on the synthesis of tetrasubstituted DKP units as polymer substrates. As commented before, there are few reports on the synthesis of such polymers, such as polyethers, peptoids, and oligoamides.[7] Recently, cell-penetrating peptidomimetics for effective DNA delivery were described.[8] These peptidomimetics were formed from DKP units containing a cationic lysine and an anionic aspartic acid. Their lateral chains were attached by peptide bonds to form the polymer backbone. However, the formation of polymers where the DKP units are bound by an inert linker, while the cationic and hydrophobic residues are free for interaction with biological targets, would be a novel approach for the synthesis of antimicrobial materials. The formation of tetrasubstituted monomers for metathesis-driven polymerization will be described herein.

Results and Discussion

Initially, we studied the scission of a model substrate **3** (Table 1), using a domino process in which the alcohol was first transformed into an *O*-radical (not shown), which underwent oxidative radical scission of the C_4–C_5 bond, forming an acyliminium ion **4**, which was trapped by acetate ions from the reagents to give the scission product **5**.[19]

Table 1. Optimization of the oxidative radical scission.



(**3**) (**4**) (**5**)

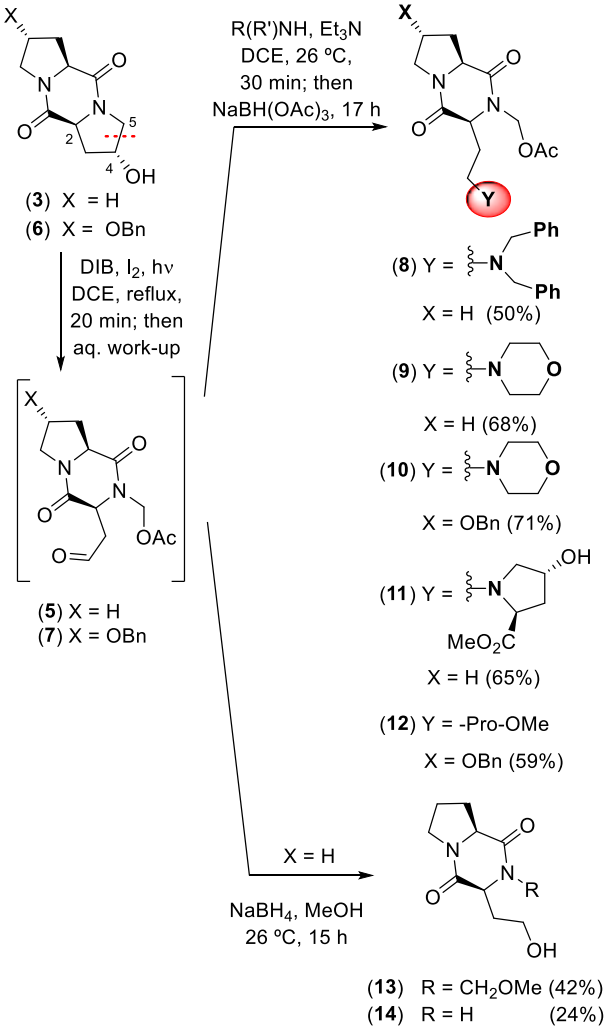
entry[a]	DIB or LTA (eq)	I_2 (Eq)	time	yield (%)[b]
1	DIB (1.5)	0.5	2 h	c.m.[c]
2	LTA (1.5)	0.5	2 h	c.m.
3	DIB (1.5)	0.3	2 h	c.m.
4	DIB (2)	0.3	30 min	15
5	DIB (2)	0.8	2 h	12
6	DIB (4)	1.5	20 min	60

[a]DIB, I_2, hν (visible), DCE, reflux; DIB = (diacetoxyiodo) benzene; LTA = lead tetraacetate; [b]Yield for products purified by chromatography. [c]c.m. = complex mixture.

The process was optimized as shown in Table 1, using different scission reagents (DIB/I_2, LTA/I_2), reagent amounts, and reaction times. In all the cases, the solvent was DCE and the mixture was irradiated with visible light.[1,20] The best conditions (entry 6) used the diacetoxyiodobenzene (DIB)-iodine system (4 and 1.5 equivalents with respect to the DKP substrate), in refluxing DCE during 20 min. Smaller amounts of DIB (entries 3-5) or iodine (entries 1-5)

resulted in complex mixtures and/or low yields of product **5**, probably because a longer reaction time was needed, which favoured side-reactions.

As shown in Scheme 1, using the optimized conditions for the oxidative radical scission, simplified two-step scission-reductive amination process (to give products **8-12**) and scission-reduction process (yielding products **13** and **14**) were implemented.



Scheme 1. Modification of Hyp customizable units in DKP.

The starting DKPs contained one or two customizable Hyp units (substrates **3** and **6**, respectively). The fragmentation products **5** or **7** were not purified; after disappearance of the starting material, the reagents were deactivated by aqueous work-up, and the organic solvent was quickly evaporated. The crude residue was then treated under reductive amination or reduction conditions, to give products **8-14** in good global yields (Scheme 1).

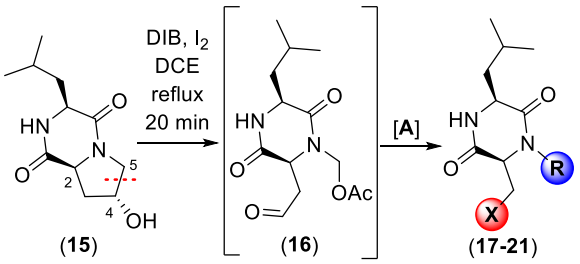
In the case of substrate **6**, two customizable Hyp units were incorporated. Since one of them was protected with a benzyl group, the scission only took place with the unprotected Hyp residue. The yields were comparable to those obtained with substrate **3** (**9** *vs.***10** and **11** *vs.***12**).

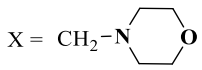
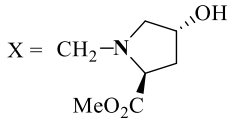
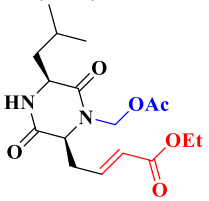
The reductive amination can provide a variety of cationic residues, from precursors of primary amines (e.g. the readily deprotected dibenzylamino group in compound **8**), to nitrogen heterocycles used in drugs (such as the morpholino group in compounds **9-10**), and extended peptides (by ligation of amino acids or peptides, as in compounds **11** and **12**).

When substrate **3** was treated under the scission-reduction protocol, two products **13** and **14** were formed (66% global yield for the two steps). Thus, the *N*-methoxymethyl derivative **13** was formed by substitution of the acetoxy group in compound **5** by the solvent (via an iminio intermediate), while compound **14** was obtained by hydrolysis of the acetate group and cleavage of the resulting hemiacetal. On larger reaction times, compound **13** can be transformed into compound **14**.

The scission and subsequent chain modification was also studied with substrate **15** (header Table 2).

Table 2. Modification of Hyp units in DKP substrate **15**.



entry	Conditions A	Products (yield)[a]
1	Bn_2NH, Et_3N, DCE, 26 ºC, 30 min; $NaBH(OAc)_3$, 17 h	**17** (59%); R = CH_2OAc X = $CH_2N(CH_2Ph)_2$
2	Morpholine, Et_3N, DCE, 26 ºC, 30 min; $NaBH(OAc)_3$, 17 h	**18** (68%) R = CH_2OAc X = 
3	H-Hyp-OMe, Et_3N, DCE, 26 ºC, 30 min; $NaBH(OAc)_3$, 17 h	**19** (52%) R = CH_2OAc X = 
4	$NaBH_4$, MeOH, 26 ºC, 15 h	**20** (57%) R = H, X = CH_2OH
6	NaH, THF, $(EtO)_2P(O)CH_2CO_2Et$	**21** (54%) 

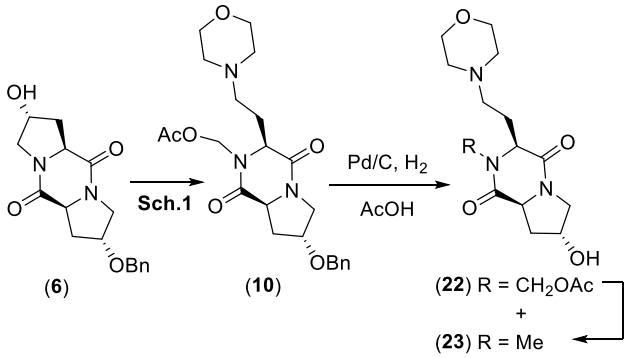
[a]Yield for products purified by chromatography.

The diketopiperazine substrate **15** not only presents a flexible α-chain, but also an unprotected NH group, which could favour side-reactions. Therefore, the aldehyde intermediate **16** was not isolated, but quickly underwent transformation under a variety of conditions [**A**] (Table 2), to afford products **17-21**.

To our satisfaction, the scission and subsequent reductive amination reactions (entries 1-3) proceeded in similar yields as before, to afford products **17-19**. However, the scission and reduction (entry 4) gave only the alcohol **20**, in which cleavage of the intermediate *N,O*-acetal was observed.

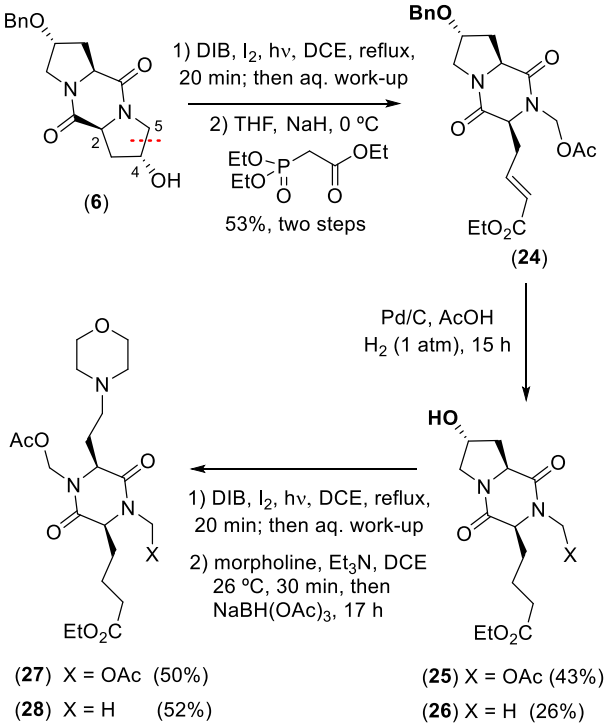
The aldehyde **16** also underwent a Horner-Wadsworth-Emmons reaction (entry 5), to afford a homoglutamic derivative **21**.[1b] The introduction of this chain allows the preparation of a variety of functionalized scaffolds, since it can be saponified and transformed into an anionic residue, or it can be transformed into esters and amides with different chains, including those containing fluorophores, branched amines, metal chelators or attached drugs.

Finally, a selective double functionalization of DKP substrates was studied, using substrate **6** (Scheme 2). As commented before, the scission only took place with the unprotected Hyp residue, and subsequent reductive amination afforded product **10**. The second Hyp unit can then be deprotected and functionalized in a different way. The cleavage of the benzyl group of compound **10** by hydrogenolysis was studied under different conditions, including different catalysts (Pd/C and $Pd(OH)_2$), amount of catalysts, solvents (MeOH, EtOAc, THF, etc), and hydrogen pressure (1-4 atm). The best results were obtained with the Pd/C system in AcOH. Under atmospheric pressure, a mixture of the *N,O*-acetal **22** (36%) and the *N*-methyl DKP **23** (28%) was obtained. When the reaction was carried out under higher hydrogen pressure (4 atm), the *N*-methyl derivative **23** was obtained as the sole product, in 67% yield.



Scheme 2. Deprotection of the second customizable Hyp unit, for a second transformation cycle.

The sequential modification of the two Hyp units to give a hydrophobic residue (homoGlu) and a cationic unit (4-aminohomoalanine) is shown in Scheme 3. Thus, substrate **6** underwent scission of the first, unprotected Hyp unit, and after aqueous work up and solvent removal, the crude reaction mixture underwent a Horner-Wittig-Emmons reaction to provide the homoglutamic derivative **24** in 53% global yield.



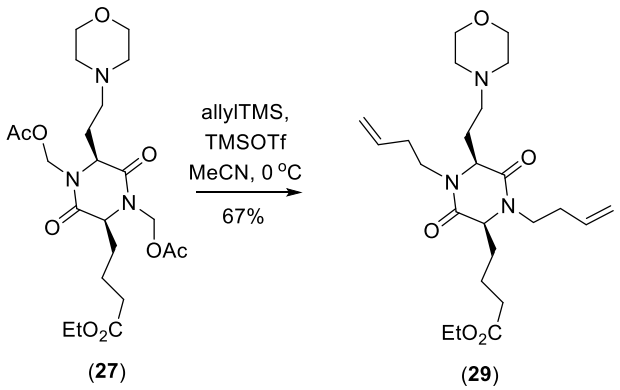
Scheme 3. Sequential transformation of two Hyp customizable units, to give products with four different ring substituents.

Then the second Hyp unit was deprotected by hydrogenolysis, accompanied by olefin reduction, to afford compound **25** and the *N*-methyl derivative **26**. Compound **25** can be quantitatively transformed into product **26** by increasing hydrogen pressure to 4 atm. A second scission took place, followed by reductive amination, that transformed compounds **25** and **26** into the DKP derivatives **27** and **28**, respectively.

The *N,O*-acetal can be further transformed into a diversity of *N*-substituents. Unlike other methodologies, where basic conditions and strong electrophiles are used to introduce the new chains, herein this step is carried out by addition of nucleophiles to the *N,O*-acetal at low temperature. Due to the mild conditions, DKP epimerization and other side-reactions are minimized.

For instance, treatment of compound **27** with allyltrimethylsilane and TMSOTf at 0 °C provided the diolefinic compound **29** (Scheme 4) in good yield. The introduction of homoallylic *N*-substituents is particularly interesting, since intermolecular metathesis followed by reduction would generate dimeric or polymeric systems with DKP units connected by inert linkers.[21] These systems would display many lateral chains for interaction with biological targets, which could enhance the bioactivity. On the other hand, the olefins can be extended (and/or "capped") by intermolecular metathesis with other olefinic compounds.

As shown with monomer (**29**), a combination of cationic and hydrophobic residues can be implemented. Moreover, the cationic character of the final molecule, and thus its bioactivity, could be modulated by transforming the hydrophobic ester into esters or amides derived from hydroxyamines, diamines, branched amines and related groups. Other functional moieties could also be attached, such as probes for molecular imaging or linkers for drug conjugation.[9]



Scheme 4. Preparation of tetrasubstituted monomers for metathesis-promoted polymerization.

The DKP scaffolds **27-29** present four different spatially-oriented chains (Figure 3). Thus, according to MM2 energy minimization studies,[22] the α-lateral chains are directed below the DKP plane, while the *N*-substituents (acetoxymethyl or allyl groups) are directed in and above the plane. In simple compounds, two chains could be used to interact with and cross biological membranes, while the other two chains can be used to shuttle conjugated drugs or probes or to provide likers in polymers.

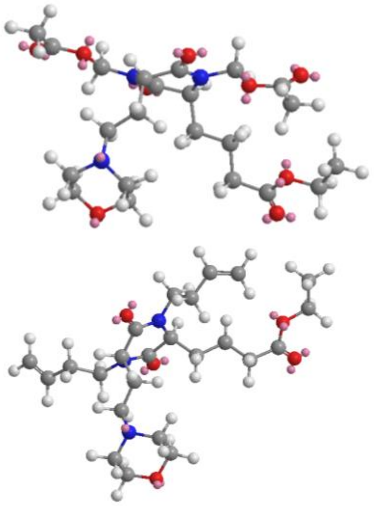


Figure 3. Tetrasubstituted DKP scaffolds for biomedical applications. MM2 for compounds (**28**, above) and (**29**).

We are currently studying the introduction of other *N*-alkyl chains and the dimerization or oligorimerization of olefinic derivatives. The bioactivity results will be reported in due course.

Conclusion

The selective manipulation of Hyp customizable units in DKP substrates allows the generation of a rigid scaffold with four new, tailor-made chains which are spatially-orientated. The key step is a domino radical scission-oxidation process which allows the generation of *N*-substituted DKPs. The chains generated after the scission can be manipulated to afford either cationic or hydrophobic residues, as required in antimicrobial SAR studies.

These compounds can be also used as monomer units for polymerization, displaying both hydrophobic and cationic chains. The preparation of a di-*N*-homoallylic substituted DKP for future metathesis-driven polymerization was illustrated. Remarkably, the bulky alkyl chains were introduced under mild conditions, by addition of nucleophiles to the intermediate *N,O*-acetals, at low temperatures. Thus, the tetrasubstituted DKP was obtained in good yield with high optical purity, meeting the requirements for antimicrobial assays.

Experimental Section

Experimental Details

General Methods. Commercially available reagents and solvents were analytical grade or were purified by standard procedures prior to use. All reactions involving air- or moisture-sensitive materials were carried out under nitrogen atmosphere. The spray reagent for TLC analysis was a potassium permanganate solution [$KMnO_4$ (10 g), K_2CO_3 (66.7 g) and NaOH (0.85 g) were dissolved in water (1L)]. Once sprayed, the TLC was heated until development of color.

Merck silica gel 60 PF_{254} and 60 (0.063-0.2 mm) were used for rotatory chromatography and column chromatography, respectively. Melting points were determined with a hot-stage apparatus and are uncorrected. Optical rotations were measured at the sodium line at ambient temperature (26 ^{o}C). Mass spectra were carried out using electrospray ionization techniques (ESI) or fast atom bombardment (FAB+) Nuclear Magnetic Resonance spectra were determined at 500 MHz for ^{1}H NMR and 125.7 MHz for ^{13}C NMR in the presence of tetramethylsilane (TMS) as internal standard, at 26 ^{o}C. ^{1}H NMR references: $CDCl_3$ (δ_H 7.26), CD_3OD (δ_H 3.31), C_6D_6 (δ_H 7.16); ^{13}C NMR references: $CDCl_3$ (δ_C 77.0), CD_3OD (δ_C 49.0), C_6D_6 (δ_C 128.4).

The amino acids H-Hyp-OMe·HCl, H-Hyp-OH, Boc-Pro-OH, H-Pro-OMe·HCl, and Boc-Leu-OH are commercial reagents and were purchased from Bachem, Fluorochem and AdvancedChemTech.

Abbreviations: br b, broad band (in the NMR spectra); DIB, (diacetoxyiodo)benzene; LTA, lead

tetraacetate; DCM, dichloromethane; DCE dichloroethane; DIPEA, diisopropylethylamine; HBTU, *O*-(benzotriazol-1-yl)-*N,N,N',N'*-tetramethyl uronium hexafluorophosphate; Hyp, hydroxyproline; THF, tetrahydrofuran.

Preparation of the Substrate Precursors

General procedure for the synthesis of 2,5-diketopiperazines: A solution of the starting dipeptide (3 mmol) was treated with acetyl chloride (2.1 mL, 2360 mg, 30 mmol) in dry methanol (10 mL) from 0 °C at 26 °C for 2 h. Then the solvent was removed under vacuum. The residue was dissolved in toluene (20 mL), treated with Et_3N (2.1 mL, 1520 mg, 15 mmol) and refluxed for 16 h. Then the solvent was removed under vacuum and the residue was purified by column chromatography yielding the 2,5-diketopiperazines.

(3*S*,6*S*)-1,6-(2*R*-hydroxytrimethylene)-3,4-(trimethylene)piperazine-2,5-dione (3) Obtained from commercial Boc-Pro-Hyp-OMe[23] (1030 mg, 3 mmol) according to the general procedure for the synthesis of 2,5-diketopiperazines. The reaction mixture was purified by column chromatography (acetone/MeOH 95:5), yielding compound (**3**) (548 mg, 87%), as a crystalline solid mp 109–111 ºC (from acetone/MeOH); $[\alpha]_D$ = −127 (c 0.45, MeOH). ^{1}H NMR (500 MHz, CD_3OD): δ_H 1.94−2.14 (m, 4H), 2.24−2.30 (m, 2H), 3.42−3.52 (m, 3H), 3.61 (dd, *J* = 4.1, 12.6 Hz, 1H), 4.38 (dd, *J* = 7.6, 8.5 Hz, 1H), 4.48 (dd, *J* = 3.8, 3.8 Hz, 1H), 4.60 (dd, *J* = 6.6, 10.7 Hz, 1H). ^{13}C NMR (125.7 MHz, CD_3OD): δ_C 24.1 (CH_2), 28.7 (CH_2), 37.7 (CH_2), 46.2 (CH_2), 54.8 (CH_2), 60.2 (CH), 61.7 (CH), 69.6 (CH), 168.7 (C), 168.8 (C). IR ($CHCl_3$) ν_{max}= 3421, 1661, 1439 cm^{-1}. HRMS (FAB$^+$) calcd for $C_{10}H_{15}N_2O_3$ (M^+ + H) 211.1083, found 211.1073. Anal. Calcd for $C_{10}H_{14}N_2O_3$: C, 57.13; H, 6.71; N, 13.33. Found: C, 56.95; H, 7.09; N, 13.33.

***N*-(*N*-*tert*-butoxycarbonyl-4*R*-benzyloxy-L-prolyl)-4*R*-hydroxy-L-proline methyl ester (30), precursor of substrate (6).** To a solution of commercial *N*-*tert*-butoxycarbonyl-4*R*-benzyloxy-L-proline (Boc-Hyp(Bn)-OH,[23] 1280 mg, 4 mmol) and 4*R*-hydroxy-L-proline methyl ester hydrochloride (H-Hyp-OMe•HCl, 872 mg, 4.8 mmol) in dry CH_2Cl_2 (40 mL) at 0 °C, was added diisopropylethylamine (1.6 mL, 1.24 g, 9.6 mmol) and *N,N,N′,N′*-tetramethyl-*O*-(1*H*-benzotriazol-1-yl)uronium hexafluorophosphate (HBTU) (1.49 g, 11 mmol). The reaction mixture was stirred for 2 h, then was washed with $NaHCO_3$ and 5% HCl, and extracted with CH_2Cl_2. The organic layer was dried on sodium sulfate, filtered and evaporated under vaccum. After purification by column chromatography (hexanes/EtOAc, 10:90), the dipeptide (**30**) was isolated (1.36 g, 76%) as a syrup; $[\alpha]_D$ = −59 (*c* 0.47, $CHCl_3$); ^{1}H NMR (500 MHz, CD_3OD): δ_H 1.40/1.44 (s/s, 9H), 1.93−2.05 (m, 2H), 2.22 (m, 1H), 2.42 (m, 1H), 3.52 (m, 1H), 3.62 (m, 1H), 3.66−3.70 (m, 2H), 3.68−3.69 (s/s, 3H), 3.74 (m, 1H), 4.22 (m, 1H), 4.46−4.52 (m, 2H), 4.53−4.57 (m, 2H), 4.60 (dd, *J* = 7.6, 7.9 Hz, 1H), 7.28 (m, 1H), 7.34 (m, 4H). ^{13}C NMR (125.7 MHz, CD_3OD): δ_C 28.7 (3 × CH_3), 36.0/36.6 (CH_2) 38.1 (CH_2), 52.7/52.9 (CH_3), 53.5 (CH_2), 55.8/56.0 (CH_2), 58.1/58.4 (CH), 59.4/59.6 (CH), 70.9/71.1 (CH), 72.1/72.2 (CH_2), 77.8/78.5 (CH), 81.6/81.8 (C), 128.8 (CH), 128.9 (2 × CH), 129.5 (2 × CH), 139.5/139.6 (C), 155.9/156.3 (C), 173.5/173.7 (C), 173.9/174.0 (C). IR ($CHCl_3$) ν_{max}= 3442, 3090, 1745, 1674, 1661, 1418 cm^{-1}. HRMS (ESI-TOF) calcd for $C_{23}H_{32}N_2O_7Na$ (M^+ + Na) 471.2107, found 471.2107. Anal. Calcd for $C_{23}H_{32}N_2O_7$: C, 61.59; H, 7.19; N, 6.25. Found C, 61.28; H, 7.45; N, 6.54.

(3*S*,6*S*)-1,6-(2*R*-benzyloxytrimethylene)-3,4-(2*R*-hydroxytrimethylene)piperazine-2,5-dione (6): Obtained from Boc-Hyp(Bn)-Hyp-OMe (**30**) (1344 mg, 3 mmol) according to the general procedure for the synthesis of 2,5-diketopiperazines. The reaction mixture was purified by column chromatography (acetone), yielding compound (**6**) (796 mg, 84%), as a foam; $[\alpha]_D$ = −147 (c 0.31, MeOH). ^{1}H NMR (500 MHz, $CDCl_3$): δ_H 2.08−2.13 (m, 2H), 2.27 (dd, *J* = 6.6, 13.6 Hz, 1H), 2.50 (dd, *J* = 6.6, 13.6 Hz, 1H), 3.44 (d, *J* = 12.6 Hz, 1H), 3.61 (m, 1H), 3.63−3.65 (m, 2H), 4.29 (m, 1H), 4.47 (m, 1H), 4.53−4.65 (m, 4H), 7.28 (m, 1H), 7.31−7.38 (m, 4H). ^{13}C NMR (125.7 MHz, MeOD): δ_C 34.9 (CH_2), 37.7 (CH_2), 52.3 (CH_2), 54.9 (CH_2), 60.1 (CH), 60.3 (CH), 69.5 (CH), 71.9 (CH_2), 77.2 (CH), 128.8 (CH), 128.9 (2 × CH), 129.5 (2 × CH), 139.3 (C), 168.7 (C), 168.9 (C). IR ($CHCl_3$) ν_{max}= 3422, 1663, 1438 cm^{-1}. HRMS (FAB$^+$) calcd for $C_{17}H_{21}N_2O_4$ (M^+ + H) 317.1501, found 317.1515. Anal. Calcd for $C_{17}H_{20}N_2O_4$: C, 64.54; H, 6.37; N, 8.86. Found: C, 64.41; H, 6.49; N, 8.95.

(3*S*,6*S*)-1,6-(2*R*-hydroxytrimethylene)-3-isobutylpiperazine-2,5-dione (15): Obtained from commercial Boc-Leu-Hyp-OMe[24] (1074 mg, 3 mmol) according to the general procedure for the synthesis of 2,5-diketopiperazines. The reaction mixture was purified by column chromatography (acetone/MeOH 95:5), yielding compound (**15**) (529 mg, 78%), as a crystalline solid mp 190–191 ºC (from acetone/MeOH); $[\alpha]_D$= −132 (c 0.21, MeOH). ^{1}H NMR (500 MHz, CD_3OD): δ_H 0.94−0.96 (m, 6H), 1.51 (m, 1H), 1.85−1.94 (m, 2H), 2.08 (m, 1H), 2.27 (dd, *J* = 6.6, 13.2 Hz, 1H), 3.43 (d, *J* = 13 Hz, 1H), 3.65 (dd, *J* = 4.4, 12.9 Hz, 1H), 4.16 (m, 1H), 4.45 (m, 1H), 4.51 (dd, *J* = 6.5, 11.0 Hz, 1H). ^{13}C NMR (125.7 MHz, CD_3OD): δ_C 22.2 (CH_3), 23.3 (CH_3), 25.8 (CH), 38.2 (CH_2), 39.4 (CH_2), 54.6 (CH), 55.2 (CH_2), 58.7 (CH), 69.1 (CH), 169.0 (C), 173.0 (C). IR ($CHCl_3$) ν_{max}= 3407, 1683, 1419 cm^{-1}. HRMS (FAB$^+$) calcd for $C_{11}H_{19}N_2O_3$ (M^+ + H) 227.1396, found 227.1383. Anal. Calcd for $C_{11}H_{18}N_2O_3$: C, 58.39; H, 8.02; N, 12.38. Found: C, 58.60; H, 7.95; N, 12.65.

Procedure for the oxidative radical scission: (3*S*,6*S*)-1-acetoxymethyl-6-(2-oxoethyl)-3,4-(trimethylene)piperazine-2,5-dione (5). A solution of the diketopiperazine **3** (42 mg, 0.2 mmol) in dry dichloroethane (4 mL) was treated with (diacetoxyiodo)benzene (258 mg, 0.8 mmol) and

iodine (76 mg, 0.3 mmol), the reaction mixture was stirred at reflux for 20 min under irradiation with visible light (80 W tungsten-filament lamp). After cooling to 26 °C the reaction mixture was poured into 10% aqueous $Na_2S_2O_3$, and extracted with CH_2Cl_2. The organic layer was dried over sodium sulfate and filtered; then the solvent was removed under vacuum, and the residue was purified by column rotatory chromatography (hexane/EtOAc 20:80), yielding compound (**5**) (32 mg, 60%), as a syrup; $[\alpha]_D$ = −66 (c 0.67, $CHCl_3$). ^{1}H NMR (500 MHz, C_6D_6): δ_H 1.01 (m, 1H), 1.14 (m, 1H), 1.55 (s, 3H), 1.78 (m, 1H), 1.88 (m, 1H), 2.58 (dd, *J* = 4.1, 17.3 Hz, 1H), 2.81 (dd, *J* = 7.3, 17.3 Hz, 1H), 2.94 (ddd, *J* = 3.8, 8.8, 12.3 Hz, 1H), 3.19–3.24 (m, 2H), 4.19 (dd, *J* = 4.4, 6.9 Hz, 1H), 5.16 (d, *J* = 10.7 Hz, 1H), 5.37 (d, *J* = 11.0 Hz, 1H), 9.43 (s, 1H). ^{13}C NMR (125.7 MHz, C_6D_6): δ_C 20.5 (CH_3), 22.8 (CH_2), 28.8 (CH_2), 41.7 (CH_2), 45.9 (CH_2), 55.8 (CH), 59.2 (CH), 67.7 (CH_2), 164.1 (C), 169.5 (C), 169.9 (C), 197.5 (C). IR ($CHCl_3$) ν_{max}= 1741, 1675, 1458 cm^{-1}. HRMS (ESI-TOF) calcd for $C_{12}H_{16}N_2O_5Na$ (M^+ + Na) 291.0957, found 291.0949. Anal. Calcd for $C_{12}H_{16}N_2O_5$: C, 53.73; H, 6.01; N, 10.44. Found: C, 53.93; H, 6.29; N, 10.38.

General procedure for the scission of 4-hydroxyproline derivatives and reductive amination with secondary amines: A solution of the substrate **3** or **6** (0.2 mmol) in dry dichloroethane (4 mL) was treated with (diacetoxyiodo)benzene (258 mg, 0.8 mmol) and iodine (76 mg, 0.3 mmol). The reaction mixture was stirred at reflux for 20 min under irradiation with visible light (80 W tungsten-filament lamp). After cooling to 26 °C the reaction mixture was poured into 10% aqueous $Na_2S_2O_3$, and extracted with CH_2Cl_2. The organic layer was dried over sodium sulfate and filtered; then the solvents was removed under vacuum. The residue was dissolved in dry dichloroethane (3 mL) and treated with the secondary amine (0.24 mmol) and triethylamine (39 µL, 0.28 mmol). After 30 min at 26 °C, sodium (triacetoxy)borohydride (127 mg, 0.6 mmol) was added. The reaction mixture was stirred for 16 h before being poured into saturated aqueous $NaHCO_3$ and extracted with CH_2Cl_2. The organic layer was dried over sodium sulfate and filtered; then the solvent was removed under vacuum, and the residue was purified by column chromatography or rotatory chromatography.

(3*S*,6*S*)-1-acetoxymethyl-6-(2-dibenzylamino ethyl)-3,4-(trimethylene)piperazine-2,5-dione (8): Obtained from substrate (**3**) **[**42 mg, 0.2 mmol] according to the general procedure for the scission of 4-hydroxyproline derivatives and reductive amination with secondary amines, using dibenzylamine (46 µL, 0.24 mmol) as the secondary amine. The reaction mixture was purified by column chromatography (CH_2Cl_2/MeOH 98:2), yielding compound (**8**) (45 mg, 50%), as a syrup; $[\alpha]_D$ = −70 (c 0.44, $CHCl_3$). ^{1}H NMR (500 MHz, CD_3OD): δ_H 1.82−1.90 (m, 3H), 1.92 (s, 3H), 2.08 (m, 1H), 2.22–2.30 (m, 2H), 2.43–2.53 (m, 2H), 3.28–3.40 (m, 2H), 3.50 (d, *J* = 13.2 Hz, 2H), 3.58 (d, *J* = 13.2 Hz, 2H), 4.09 (dd, *J* = 6.6, 7.9 Hz, 1H), 4.29 (dd, *J* = 1.9, 6.5 Hz, 1H), 5.13 (d, *J* = 10.7 Hz, 1H), 5.48 (d, *J* = 10.7 Hz, 1H), 7.21 (dd, *J* = 5.7, 6.9 Hz, 2H), 7.26−7.37 (m, 8H). ^{13}C NMR (125.7 MHz, CD_3OD): δ_C 20.7 (CH_3), 23.3 (CH_2), 26.6 (CH_2), 29.5 (CH_2), 46.1 (CH_2), 49.7 (CH_2), 58.4 (CH), 59.6 (2 × CH_2), 60.2 (CH), 68.0 (CH_2), 128.1 (2 × CH), 129.3 (4 × CH), 130.2 (4 × CH), 140.8 (2 × C), 166.7 (C), 171.7 (C), 171.8 (C). IR ($CHCl_3$) ν_{max}= 1741, 1671, 1453 cm^{-1}. HRMS (FAB^+) calcd for $C_{26}H_{32}N_3O_4$ (M^+ + H) 450.2393, found 450.2409. Anal. Calcd for $C_{26}H_{31}N_3O_4$: C, 69.47; H, 6.95; N, 9.35. Found: C, 69.55; H, 6.88; N, 9.52.

(3*S*,6*S*)-1-acetoxymethyl-6-(2-morpholinoethyl)-3,4-(trimethylene)piperazine-2,5-dione (9): Obtained from substrate (**3**) **[**42 mg, 0.2 mmol] according to the general procedure for the scission of 4-hydroxyproline derivatives and reductive amination with secondary amines, using morpholine (21 µL, 0.24 mmol) as the secondary amine. The reaction mixture was purified by column chromatography (CH_2Cl_2/MeOH 98:2), yielding compound (**9**) (48 mg, 68%) as a syrup; $[\alpha]_D$ = −40 (c 0.14, $CHCl_3$). ^{1}H NMR (500 MHz, CD_3OD): δ_H 1.90−2.00 (m, 2H), 2.03 (m, 1H), 2.05 (s, 3H), 2.14 (m, 1H), 2.34–2.43 (m, 6H), 2.45–2.55 (m, 2H), 3.47 (ddd, *J* = 3.5, 8.8, 12.0 Hz, 1H), 3.65−3.69 (m, 5H), 4.25 (dd, *J* = 6.3, 8.5 Hz, 1H), 4.40 (m, 1H), 5.39 (d, *J* = 10.7 Hz, 1H), 5.68 (d, *J* = 10.7 Hz, 1H). ^{13}C NMR (125.7 MHz, CD_3OD): δ_C 20.6 (CH_3), 22.9 (CH_2), 26.2 (CH_2), 30.1 (CH_2), 46.4 (CH_2), 54.9 (2 × CH_2), 55.0 (CH_2), 59.2 (CH), 60.2 (CH), 67.8 (2 × CH_2), 68.8 (CH_2), 166.3 (C), 171.1 (C), 171.9 (C). IR ($CHCl_3$) ν_{max}= 1742, 1667, 1449 cm^{-1}. HRMS (ESI-TOF) calcd for $C_{16}H_{26}N_3O_5$ (M^+ + H) 340.1872, found 340.1870. Anal. Calcd for $C_{16}H_{25}N_3O_5$: C, 56.62; H, 7.43; N, 12.38. Found: C, 56.48; H, 7.55; N, 12.58.

(3*S*,6*S*)-1-acetoxymethyl-6-(2-morpholinoethyl)-3,4-(2*R*-benzyloxytrimethylene)piperazine-2,5-dione (10): Obtained from substrate (**6**) **[**63 mg, 0.2 mmol] according to the general procedure for the scission of 4-hydroxyproline derivatives and reductive amination with secondary amines, using morpholine (21 µL, 0.24 mmol) as the secondary amine. The reaction mixture was purified by column chromatography (CH_2Cl_2/MeOH 98:2), yielding compound (**10**) (63 mg, 71%), a yellowish syrup; $[\alpha]_D$ = −43 (c 0.43, $CHCl_3$). ^{1}H NMR (500 MHz, CD_3OD): δ_H 2.00 (m, 1H), 2.04 (m, 3H), 2.14 (m, 1H), 2.36–2.43 (m, 5H), 2.44–2.50 (m, 2H), 2.56 (dd, *J* = 6.0, 13.2 Hz, 1H), 3.56 (d, *J* = 13.2 Hz, 1H), 3.63 (dd, *J* = 4.4, 4.4 Hz, 4H), 3.85 (dd, *J* = 5.0, 13.2 Hz, 1H), 4.28 (dd, *J* = 4.7, 4.7 Hz, 1H), 4.41 (m, 1H), 4.48 (dd, *J* = 6.0, 11.4 Hz, 1H), 4.54 (d, *J* = 11.7 Hz, 1H), 4.58 (d, *J* = 11.7 Hz, 1H), 5.37 (d, *J* = 10.7 Hz, 1H), 5.69 (d, *J* = 10.7 Hz, 1H), 7.27 (m, 1H), 7.31−7.38 (m, 4H). ^{13}C NMR (125.7 MHz, CD_3OD): δ_C 20.7 (CH_3), 26.1 (CH_2), 36.4 (CH_2), 53.3 (CH_2), 54.8 (2 × CH_2), 55.0 (CH_2), 58.6 (CH), 59.0 (CH), 67.7 (2 × CH_2), 68.7 (CH), 72.0 (CH_2), 76.3 (CH_2), 128.8 (CH), 128.9 (2 × CH), 129.5 (2 × CH), 139.3

(C), 166.3 (C), 171.3 (C), 171.9 (C). IR ($CHCl_3$) ν_{max}= 1742, 1671, 1456 cm^{-1}. HRMS (FAB^+) calcd for $C_{23}H_{32}N_3O_6$ (M^+ + H) 446.2291, found 446.2279. Anal. Calcd for $C_{23}H_{31}N_3O_6$: C, 62.01; H, 7.01; N, 9.43. Found: C, 61.91; H, 7.21; N, 9.16.

(3*S*,6*S*)-1-acetoxymethyl-6-(2-[4*R*-hydroxy-2*S*-methoxycarbonylpyrrolidinyl]ethyl)-3,4-(trimethylene)piperazine-2,5-dione (11): Obtained from substrate (**3**) **[**42 mg, 0.2 mmol] according to the general procedure for the scission of 4-hydroxyproline derivatives and reductive amination with secondary amines, using H-Hyp-OMe·HCl (44 mg, 0.24 mmol) as the secondary amine. The reaction mixture was purified by column chromatography (CH_2Cl_2/MeOH 98:2), yielding compound (**11**) (52 mg, 65%) as a syrup; $[\alpha]_D$ = −5 (c 0.15, MeOH). ^{1}H NMR (500 MHz, $CDCl_3$): δ_H 1.85−2.03 (m, 3H), 2.06 (s, 3H), 2.12−2.24 (m, 2H), 2.31−2.42 (m, 3H), 2.58 (dd, *J* = 4.1, 10.1 Hz, 1H), 2.65 (m, 1H), 2.92 (m, 1H), 3.26 (d, *J* = 9.8 Hz, 1H), 3.34 (dd, *J* = 4.4, 9.8 Hz, 1H), 3.49 (ddd, *J* = 3.8, 9.1, 12.3 Hz, 1H), 3.65 (m, 1H), 3.70 (s, 3H), 4.14 (dd, *J* = 7.6, 8.5 Hz, 1H), 4.28 (m, 1H), 4.38 (br d, *J* = 6.3 Hz, 1H), 5.23 (d, *J* = 10.7 Hz, 1H), 5.82 (d, *J* = 10.7 Hz, 1H). ^{13}C NMR (125.7 MHz, $CDCl_3$): δ_C 20.8 (CH_3), 22.4 (CH_2), 26.0 (CH_2), 28.4 (CH_2), 38.9 (CH_2), 45.5 (CH_2), 49.8 (CH_2), 52.3 (CH_2), 56.5 (CH_3), 59.1 (CH), 62.0 (CH), 64.1 (CH), 66.9 (CH), 70.3 (CH_2), 164.8 (C), 169.9 (C), 170.3 (C), 174.3 (C). IR ($CHCl_3$) ν_{max}= 3143, 1742, 1673, 1439 cm^{-1}. HRMS (FAB^+) calcd for $C_{18}H_{28}N_3O_7$ (M^+ + H) 398.1927, found 398.1944. Anal. Calcd for $C_{18}H_{27}N_3O_7$: C, 54.40; H, 6.85; N, 10.57. Found: C, 54.40; H, 6.98; N, 10.22.

(3*S*,6*S*)-1-acetoxymethyl-6-(2-[4*R*-hydroxy-2*S*-methoxycarbonylpyrrolidinyl]ethyl)-3,4-(2*R*-benzyloxytrimethylene)piperazine-2,5-dione (12): Obtained from substrate (**6**) **[**63 mg, 0.2 mmol] according to the general procedure for the scission of 4-hydroxyproline derivatives and reductive amination with secondary amines, using H-Pro-OMe·HCl (58 mg, 0.24 mmol) as the secondary amine. The reaction mixture was purified by column chromatography (CH_2Cl_2/MeOH 98:2), yielding compound (**12**) (57 mg, 59%) as a yellowish syrup; $[\alpha]_D$ = −68 (c 0.20, $CHCl_3$). ^{1}H NMR (500 MHz, CD_3OD): δ_H 1.78−1.92 (m, 3H), 2.04 (m, 1H), 2.06 (s, 3H), 2.14 (m, 1H), 2.20 (m, 1H), 2.34−2.43 (m, 3H), 2.55 (dd, *J* = 6.0, 13.2 Hz, 1H), 2.76 (m, 1H), 3.13 (m, 1H), 3.20 (m, 1H), 3.57 (d, *J* = 13.2 Hz, 1H) 3.69 (s, 3H), 3.78 (dd, *J* = 5.0, 13.2 Hz, 1H), 4.26 (dd, *J* = 4.4, 4.7 Hz, 1H), 4.43−4.50 (m, 2H), 4.54 (d, *J* = 11.7 Hz, 1H), 4.58 (d, *J* = 11.7 Hz, 1H), 5.37 (d, *J* = 10.7 Hz, 1H), 5.70 (d, *J* = 10.7 Hz, 1H), 7.27 (m, 1H), 7.31−7.37 (m, 4H). ^{13}C NMR (125.7 MHz, CD_3OD): δ_C 20.7 (CH_3), 23.9 (CH_2), 27.9 (CH_2), 30.0 (CH_2), 36.2 (CH_2), 50.9 (CH_2), 52.3 (CH_3), 53.1 (CH_2), 54.2 (CH_2), 58.6 (CH), 59.0 (CH), 67.0 (CH), 68.7 (CH_2), 71.9 (CH), 76.3 (CH_2), 128.8 (CH), 128.9 (2 × CH), 129.4 (2 × CH), 139.3 (C), 166.3 (C), 171.5 (C), 171.9 (C), 175.7 (C). IR ($CHCl_3$) ν_{max}= 3066, 1741, 1673, 1456, 1438 cm^{-1}. HRMS (ESI-TOF) calcd for $C_{25}H_{33}N_3O_7Na$ (M^+ + Na) 510.2216, found 510.2215. Anal. Calcd for $C_{25}H_{33}N_3O_7$: C, 61.59; H, 6.82; N, 8.62. Found: C, 61.88; H, 7.16; N, 8.62.

Procedure for the scission of 4-hydroxyproline derivatives and reduction process: A solution of the substrate (**3**) **[**42 mg, 0.2 mmol] in dry dichloroethane (4 mL) was treated with (diacetoxyiodo)benzene (258.0 mg, 0.8 mmol) and iodine (76.0 mg, 0.3 mmol). The reaction mixture was stirred at reflux for 20 min under irradiation with visible light (80 W tungsten-filament lamp). After cooling to 26 °C the reaction mixture was poured into 10% aqueous $Na_2S_2O_3$, and extracted with CH_2Cl_2. The organic layer was dried over sodium sulfate and filtered; then the solvent was removed under vacuum. The residue was dissolved in dry methanol (2 mL) and sodium borohydride (11 mg, 0.3 mmol) was added. The reaction mixture was stirred for 16 h, before removing the solvent under vacuum. The residue was purified by rotatory chromatography (CH_2Cl_2/MeOH 98:2), to afford the alcohols (**13**, 20 mg, 42%) and (**14**, 9 mg, 24%).

(3*S*,6*S*)-1-methoxymethyl-6-(2-hydroxyethyl)-3,4-(trimethylene)piperazine-2,5-dione (13): as a syrup; $[\alpha]_D$ = −49 (c 0.38, $CHCl_3$). ^{1}H NMR (500 MHz, CD_3OD): δ_H 1.90−2.10 (m, 3H), 2.23 (m, 1H), 2.31−2.45 (m, 2H), 3.30 (s, 3H), 3.47 (ddd, *J* = 3.8, 9.1, 12.3 Hz, 1H), 3.60−3.70 (m, 3H), 4.28−4.34 (m, 2H), 4.74 (d, *J* = 10.4 Hz, 1H), 5.12 (d, *J* = 10.4 Hz, 1H). ^{13}C NMR (125.7 MHz, CD_3OD): δ_C 23.3 (CH_2), 29.7 (CH_2), 31.7 (CH_2), 46.5 (CH_2), 56.7 (CH_3), 57.5 (CH), 59.2 (CH_2), 60.2 (CH), 74.9 (CH_2), 167.6 (C), 171.9 (C). IR ($CHCl_3$) ν_{max}= 3446, 1665, 1452 cm^{-1}. HRMS (FAB^+) calcd for $C_{11}H_{19}N_2O_4$ (M^+ + H) 243.1345, found 243.1356. Anal. Calcd for $C_{11}H_{18}N_2O_4$: C, 54.53; H, 7.49; N, 11.56. Found: C, 54.76; H, 7.83; N, 11.32.

(3*S*,6*S*)-6-(2-hydroxyethyl)-3,4-(trimethylene)piperazine-2,5-dione (14): $[\alpha]_D$ = −90 (c 0.15, $CHCl_3$). ^{1}H NMR (500 MHz, CD_3OD): δ_H 1.84−1.95 (m, 2H), 1.97−2.05 (m, 2H), 2.23 (m, 1H), 2.30 (m, 1H), 3.50−3.53 (m, 2H), 3.71−3.84 (m, 2H), 4.23−4.26 (m, 2H). ^{13}C NMR (125.7 MHz, CD_3OD): δ_C 23.5 (CH_2), 29.1 (CH_2), 33.1 (CH_2), 46.5 (CH_2), 55.3 (CH), 60.18 (CH), 60.22 (CH_2), 168.3 (C), 172.5 (C). IR ($CHCl_3$) ν_{max}= 3351, 1667, 1430 cm^{-1}. HRMS (FAB^+) calcd for $C_9H_{15}N_2O_3$ (M^+ + H) 199.1083, found 199.1071. Anal. Calcd for $C_9H_{14}N_2O_3$: C, 54.53; H, 7.12; N, 14.13. Found: C, 54.59; H, 6.95; N, 14.29.

(3*S*,6*S*)-1-(acetoxymethyl)-6-(2-(dibenzylamino) ethyl)-3-isobutylpiperazine-2,5-dione (17): Formed from diketopiperazine (**15**) **[**45 mg, 0.2 mmol] according to the general procedure for the scission of 4-hydroxyproline derivatives and reductive amination with secondary amines, using dibenzylamine (46 µL, 0.24 mmol) as secondary amines. The reaction mixture was purified by column chromatography (CH_2Cl_2/MeOH 98:2), yielding compound (**17**) (55 mg, 59%), as a syrup; $[\alpha]_D$ = −31 (c 0.24, $CHCl_3$). ^{1}H NMR (500 MHz, CD_3OD): δ_H 0.91 (d, *J* = 6.6 Hz, 3H), 0.93 (d, *J* = 6.5 Hz, 3H), 1.48 (m, 1H), 1.56 (m, 1H), 1.74 (m, 1H), 1.98 (s, 3H), 2.06−2.10 (m, 2H),

2.56 (m, 1H), 2.65 (m, 1H), 3.48 (d, *J* = 13.6 Hz, 2H), 3.69 (d, *J* = 13.6 Hz, 2H), 3.91 (dd, *J* = 5.0, 9.5 Hz, 1H), 4.20 (dd, *J* = 5.7, 6.0 Hz, 1H), 5.06 (d, *J* = 10.4 Hz, 1H), 5.22 (d, *J* = 10.4 Hz, 1H), 7.22 (dd, *J* = 7.3, 7.3 Hz, 2H), 7.29 (dd, *J* = 7.3, 7.6 Hz, 4H), 7.35 (m, 4H). ^{13}C NMR (125.7 MHz, CD_3OD): δ_C 20.7 (CH_3), 21.7 (CH_3), 23.5 (CH_3), 25.4 (CH), 33.0 (CH_2), 46.4 (CH_2), 50.5 (CH_2), 54.9 (CH), 59.3 (2 × CH_2), 59.9 (CH), 70.6 (CH_2), 128.2 (2 × CH), 129.4 (2 × CH), 129.5 (2 × CH), 130.2 (4 × CH), 140.4 (2 × C), 169.8 (C), 170.3 (C), 171.9 (C). IR ($CHCl_3$) ν_{max}= 3399, 1744, 1683, 1456 cm^{-1}. HRMS (FAB$^+$) calcd for $C_{27}H_{36}N_3O_4$ (M^+ + H) 466.2706, found 466.2732. Anal. Calcd for $C_{27}H_{35}N_3O_4$: C, 69.65; H, 7.58; N, 9.03. Found: C, 69.33; H, 7.89; N, 8.77.

(3*S*,6*S*)-1-acetoxymethyl-6-(2-morpholinoethyl)-3-isobutylpiperazine-2,5-dione (18): Formed from diketopiperazine (**15**) **[**45 mg, 0.2 mmol] according to the general procedure for the scission of 4-hydroxyproline derivatives and reductive amination with secondary amines, using morpholine (21 µL, 0.24 mmol) as amine. The reaction mixture was purified by column chromatography (CH_2Cl_2/MeOH 98:2), yielding compound (**18**) (48 mg, 68%), as a syrup; $[\alpha]_D$ = −18 (c 0.17, $CHCl_3$). ^{1}H NMR (500 MHz, CD_3OD): δ_H 0.97 (d, *J* = 6.3 Hz, 3H), 1.00 (d, *J* = 6.6 Hz, 3H), 1.64−1.72 (m, 2H), 1.83 (m, 1H), 2.07 (s, 3H), 2.16 (m, 1H), 2.08−2.09 (m, 1H), 2.46−2.58 (m, 6H), 3.69 (dd, *J* = 4.7, 4.7 Hz, 4H), 4.00 (dd, *J* = 5.4, 9.1 Hz, 1H), 4.26 (dd, *J* = 5.4, 7.3 Hz, 1H), 5.45 (d, *J* = 10.3 Hz, 1H), 5.49 (d, *J* = 10.3 Hz, 1H). ^{13}C NMR (125.7 MHz, CD_3OD): δ_C 20.7 (CH_3), 21.7 (CH_3), 23.5 (CH_3), 25.4 (CH), 32.0 (CH_2), 46.4 (CH_2), 54.6 (2 × CH_2), 55.0 (CH_2), 55.4 (CH), 59.7 (CH), 67.7 (2 × CH_2), 70.7 (CH_2), 169.8 (C), 170.5 (C), 172.1 (C). IR ($CHCl_3$) ν_{max}= 3399, 1743, 1683, 1458 cm^{-1}. HRMS (ESI-TOF) calcd for $C_{17}H_{29}N_3O_5Na$ (M^+ + Na) 378.2005, found 378.2008. Anal. Calcd for $C_{17}H_{29}N_3O_5$: C, 57.45; H, 8.22; N, 11.82. Found: C, 57.62; H, 8.08; N, 11.59.

(3*S*,6*S*)-1-acetoxymethyl-6-(2-[4*R*-hydroxy-2*S*-methoxycarbonylpyrrolidinyl]ethyl)-3-isobutyl piperazine-2,5-dione (19): Obtained from diketopiperazine (**15**) **[**45 mg, 0.2 mmol] according to the general procedure for the scission of 4-hydroxyproline derivatives and reductive amination with secondary amines, using H-Hyp-OMe·HCl (44 mg, 0.24 mmol) as secondary amines. The reaction mixture was purified by column chromatography (CH_2Cl_2/MeOH 98:2), yielding compound (**19**) (43 mg, 52%), as a syrup; $[\alpha]_D$ = −41 (c 0.10, MeOH). ^{1}H NMR (500 MHz, CD_3OD): δ_H 0.96 (d, *J* = 6.6 Hz, 3H), 0.99 (d, *J* = 6.6 Hz, 3H), 1.61−1.73 (m, 2H), 1.83 (m, 1H), 2.00−2.14 (m, 3H), 2.07 (s, 3H), 2.40 (dd, *J* = 4.1, 10.1 Hz, 1H), 2.58 (m, 1H), 2.93 (m, 1H), 3.42 (dd, *J* = 5.7, 10.2 Hz, 1H), 3.51 (dd, *J* = 7.9, 8.2 Hz, 1H), 3.69 (s, 3H), 3.98 (dd, *J* = 5.4, 9.1 Hz, 1H), 4.27 (dd, *J* = 5.4, 6.9 Hz, 1H), 4.36 (m, 1H), 5.36 (d, *J* = 10.4 Hz, 1H), 5.55 (d, *J* = 10.4 Hz, 1H). ^{13}C NMR (125.7 MHz, CD_3OD): δ_C 20.7 (CH_3), 21.8 (CH_3), 23.5 (CH_3), 25.4 (CH), 34.3 (CH_2), 40.1 (CH_2), 46.4 (CH_2), 52.1 (CH_2), 52.4 (CH_3), 55.0 (CH), 59.9 (CH_2), 62.2 (CH), 66.0 (CH), 70.6 (CH), 71.2 (CH_2), 169.8 (C), 170.5 (C), 172.1 (C) 175.5 (C). IR ($CHCl_3$) ν_{max}= 3390, 1740, 1683, 1438 cm^{-1}. HRMS (ESI-TOF) calcd for $C_{19}H_{31}N_3O_7Na$ (M^+ + Na) 436.2060, found 436.2051. Anal. Calcd for $C_{19}H_{31}N_3O_7$: C, 55.19; H, 7.56; N, 10.16. Found: C, 55.59; H, 7.19; N, 10.15.

(3S,6S)-6-(2-hydroxyethyl)-3-isobutylpiperazine-2,5-dione (20): Obtained from diketopiperazine (**15**) **[**45 mg, 0.2 mmol] according to the general procedure for the scission of 4-hydroxyproline derivatives and reduction. The reaction mixture was purified by rotatory chromatography (CH_2Cl_2/MeOH 99:1), yielding compound (**20**) as an amorphous solid (24 mg, 57%): $[\alpha]_D$ = −25 (c 0.21, MeOH). ^{1}H NMR (500 MHz, CD_3OD): δ_H 0.95 (d, *J* = 6.6 Hz, 3H), 0.97 (d, *J* = 6.6 Hz, 3H) 1.64 (m, 1H), 1.72 (m, 1H), 1.84 (m, 1H), 1.91 (m, 1H), 2.12 (m, 1H), 3.71−3.74 (m, 2H), 3.93 (dd, *J* = 4.4, 8.5 Hz, 1H), 4.05 (dd, *J* = 4.7, 8.2 Hz, 1H). ^{13}C NMR (125.7 MHz, CD_3OD): δ_C 22.0 (CH_3), 23.5 (CH_3), 25.3 (CH), 38.3 (CH_2), 45.2 (CH_2), 54.0 (CH), 54.6 (CH), 59.3 (CH_2), 170.8 (C), 171.2 (C). IR ($CHCl_3$): 3393, 3354, 1678, 1451 cm^{-1}. HRMS (FAB$^+$) calcd for $C_{10}H_{19}N_2O_3$ (M^+ + H) 215.1396, found 215.1414. Anal. Calcd for $C_{10}H_{18}N_2O_3$: C, 56.06; H, 8.47; N, 13.07. Found: C, 56.23; H, 8.43; N, 12.75.

Procedure for the scission and Horner-Wadsworth-Emmons reaction. Preparation of (3*S*,6*S*)-1-(acetoxymethyl)-6-(3-ethoxycarbonyl-2-propen-1-yl)-3-isobutyl piperazine-2,5-dione (21): A solution of the substrate (**15**) **[**45 mg, 0.2 mmol] in dry dichloroethane (4 mL) was treated with (diacetoxyiodo)benzene (258.0 mg, 0.8 mmol) and iodine (76.0 mg, 0.3 mmol). The reaction mixture was stirred at reflux for 20 min under irradiation with visible light (80 W tungsten-filament lamp), and then was poured into 10% aqueous $Na_2S_2O_3$, and extracted with CH_2Cl_2. The organic layer was dried over sodium sulfate and filtered; the solvent was removed under vacuum, to give a residue containing the scission product (aldehyde), which was not purified, but was used directly in a Horner-Wadsworth-Emmons (HWE) reaction.

The HWE reagent was prepared from triethyl phosphonoacetate (44 μ L, 49.7 mg, 0.22 mmol) which was slowly added to a suspension of sodium hydride (60% in mineral oil, 9 mg, 0.22 mmol) in dry THF (3mL) at −20 °C, and stirred for 1 h. Then, a solution of the crude aldehyde in dry THF (2 mL) was added dropwise to the HWE reagent, and the mixture was stirred at −20 °C for 2 h before being poured into water and extracted with diethyl ether. The organic layer was dried, filtered and evaporated as usual, and the residue was purified by rotatory chromatography (CH_2Cl_2/MeOH, 99:1), yielding product (**21**) (38 mg, 54%) as a yellowish syrup; $[\alpha]_D$ = −48 (c 0.08, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$): δ_H 0.93 (d, *J* = 5.7 Hz, 3H), 0.96 (d, *J* = 6.0 Hz, 3H), 1.27 (dd, *J* = 7.0, 7.3 Hz, 3H), 1.59 (m, 1H), 1.70−1.80 (m, 2H), 2.09 (s, 3H), 2.82 (m, 1H), 2.92 (m, 1H), 4.03 (m, 1H), 4.17 (ddd, *J* = 7.0, 7.3, 7.5 Hz, 2H), 4.31 (dd, *J* = 4.7, 5.0 Hz, 1H), 5.39 (d, *J* = 10.4

Hz, 1H), 5.50 (d, *J* = 10.4 Hz, 1H), 5.93 (d, *J* = 15.8 Hz, 1H), 6.83 (ddd, *J* = 7.6, 7.9, 15.5 Hz, 1H), 7.38 (br b, 1H). ^{13}C NMR (125.7 MHz, $CDCl_3$): δ_C 14.2 (CH_3), 20.7 (CH_3), 20.8 (CH_3), 23.0 (CH_3), 24.2 (CH), 35.2 (CH_2), 45.0 (CH_2), 53.7 (CH), 59.0 (CH), 60.6 (CH_2), 68.3 (CH_2), 126.4 (CH), 140.7 (CH), 165.3 (C), 166.4 (C), 167.6 (C), 170.5 (C). IR ($CHCl_3$) ν_{max}= 3394, 1744, 1716, 1684, 1457 cm^{-1}. HRMS (ESI-TOF) calcd for $C_{17}H_{26}N_2O_6Na$ (M^+ + Na) 377.1689, found 377.1684. Anal. Calcd for $C_{17}H_{26}N_2O_6$: C, 57.61; H, 7.39; N, 7.90. Found: C, 57.42; H, 7.44; N, 8.17.

Procedure for deprotection of the benzyl ether in diketopiperazines. Preparation of (3*S*,6*S*)-1-acetoxymethyl-6-(2-morpholinoethyl)-3,4-(2*R*-hydroxytrimethylene)piperazine-2,5-dione (22) and (3*S*,6*S*)-1-methyl-6-(2-morpholinoethyl)-3,4-(2*R*-hydroxytrimethylene)piperazine-2,5-dione (23): A solution of the benzyl ether derivative, such as substrate **10** (67 mg, 0.15 mmol), in acetic acid (2.5 mL) was treated with Pd (10% on carbon, 25 mg) and the reaction was stirred at room temperature and under hydrogen atmosphere (1 atm) for 16 h. Then the mixture was filtered through Celite and the solvent was removed under vacuum. The residue was purified by rotatory chromatography (CH_2Cl_2/MeOH 96:4), yielding compounds (**22**, 19 mg, 36%) and (**23**, 12.5 mg, 28%). When the reaction was performed under higher hydrogen pressure (4 atm), compound **23** was obtained (30 mg, 67%).

Compound (**22**): Syrup; $[\alpha]_D$ = −51 (c 0.08, MeOH). ^{1}H NMR (500 MHz, $CDCl_3$): δ_H 2.02–2.15 (m, 2H), 2.05 (m, 3H), 2.37–2.41 (m, 6H), 2.48–2.55 (m, 2H), 3.47 (d, *J* = 12.9 Hz, 1H), 3.60–3.68 (m, 4H), 3.75–3.88 (m, 2H), 4.30 (d, *J* = 6.0 Hz, 1H), 4.50 (m, 1H), 4.54 (dd, *J* = 4.1, 4.1 Hz, 1H), 5.24 (d, *J* = 10.7 Hz, 1H), 5.79 (d, *J* = 10.7 Hz, 1H). ^{13}C NMR (125.7 MHz, $CDCl_3$): 20.7 (CH_3), 24.5 (CH_2), 38.5 (CH_2), 53.5 (2 × CH_2), 53.9 (CH_2), 54.6 (CH_2), 57.20 (CH), 57.24 (CH), 66.5 (2 × CH_2), 66.9 (CH), 67.8 (CH_2), 164.3 (C), 169.7 (C), 170.2 (C). IR ($CHCl_3$) ν_{max}= 3422, 1742, 1672, 1446 cm^{-1}. HRMS (ESI-TOF) calcd for $C_{16}H_{26}N_3O_6$ (M^+ + H) 356.1822, found 356.1819. Anal. Calcd for $C_{16}H_{25}N_3O_6$: C, 54.07; H, 7.09; N, 11.82. Found: C, 54.29; H, 7.19; N, 11.89.

Compound (**23**): Syrup; $[\alpha]_D$ = −25 (c 0.39, MeOH). ^{1}H NMR (500 MHz, CD_3OD): δ_H 1.90 (ddd, *J* = 4.4, 12.3, 12.6 Hz, 1H), 2.09 (m, 1H), 2.23–2.28 (m, 2H), 2.28–2.35 (m, 3H), 2.42–2.54 (m, 3H), 2.99 (s, 3H), 3.32 (d, *J* = 13.2 Hz, 1H), 3.58–3.66 (m, 4H), 3.95 (dd, *J* = 5.4, 13.2 Hz, 1H), 4.25 (m, 1H), 4.43–4.48 (m, 2H). ^{13}C NMR (125.7 MHz, CD_3OD): δ_C 26.5 (CH_2), 31.8 (CH_3), 40.3 (CH_2), 54.6 (CH_2), 54.9 (2 × CH_2), 56.0 (CH_2), 58.2 (CH), 61.0 (CH), 67.7 (2 × CH_2), 68.4 (CH), 166.1 (C), 169.5 (C). IR ($CHCl_3$) ν_{max}= 3421, 1657, 1460 cm^{-1}. HRMS (ESI-TOF) calcd for $C_{14}H_{24}N_3O_4$ (M^+ + H) 298.1767, found 298.1761. Anal. Calcd for $C_{14}H_{23}N_3O_4$: C, 56.55; H, 7.80; N, 14.13. Found: C, 56.34; H, 8.14; N, 13.85.

(3*S*,6*S*)-1-acetoxymethyl-6-(4-ethoxy-4-oxo-2-butenyl)-3,4-(2*R*-benzyloxytrimethylene) piperazine-2,5-dione (24): Obtained from substrate **6** [315 mg, 1 mmol] according to the general procedure for the scission of 4-hydroxyproline derivatives and HWE procedure, extrapolating (5-fold) the general amounts. After work-up, the residue was purified by column chromatography (hexanes/AcOEt, 1:1), yielding product (**24**) (236 mg, 53%) as a yellowish syrup; $[\alpha]_D$ = −63 (c 0.42, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$): δ_H 1.26 (dd, *J* = 7.0, 7.3 Hz, 3H), 2.03 (m, 1H), 2.07 (s, 3H), 2.64 (dd, *J* = 6.0, 13.6 Hz, 1H), 2.94 (m, 1H), 3.17 (m, 1H), 3.68 (d, *J* = 13.2 Hz, 1H), 3.83 (dd, *J* = 5.0, 13.6 Hz, 1H), 4.16 (ddd, *J* = 7.0, 7.0, 7.3 Hz, 2H), 4.23 (dd, *J* = 4.4, 4.4 Hz, 1H), 4.37 (m, 1H), 4.46 (ddd, *J* = 5.8, 6.3, 6.7 Hz, 1H), 4.51 (d, *J* = 11.7 Hz, 1H), 4.55 (d, *J* = 11.7 Hz, 1H), 5.22 (d, *J* = 10.7 Hz, 1H), 5.77 (d, *J* = 10.7 Hz, 1H), 5.96 (d, *J* = 15.8 Hz, 1H), 6.77 (m, 1H), 7.26–7.40 (m, 5H). ^{13}C NMR (125.7 MHz, $CDCl_3$): δ_C 14.2 (CH_3), 20.7 (CH_3), 31.1 (CH_2), 35.4 (CH_2), 52.0 (CH_2), 57.4 (CH), 58.1 (CH), 60.5 (CH_2), 67.0 (CH), 71.0 (CH_2), 74.7 (CH_2), 125.3 (CH), 127.7 (2 × CH), 128.0 (CH), 128.6 (2 × CH), 137.2 (C), 142.1 (CH), 163.1 (C), 165.7 (C), 169.3 (C), 170.3 (C). IR ($CHCl_3$) ν_{max}= 3090, 3066, 1742, 1710, 1676, 1456 cm^{-1}. HRMS (ESI-TOF) calcd for $C_{23}H_{28}N_2O_7Na$ (M^+ + Na) 467.1794, found 467.1793. Anal. Calcd for $C_{23}H_{28}N_2O_7$: C, 62.15; H, 6.35; N, 6.30. Found: C, 62.23; H, 6.38; N, 6.51.

(3*S*,6*S*)-1-acetoxymethyl-6-(4-ethoxy-4-oxo-2-butenyl)-3,4-(2*R*-hydroxytrimethylene)piperazine-2,5-dione (25) and (3*S*,6*S*)-1-methyl-6-(4-ethoxy-4-oxo-2-butenyl)-3,4-(2*R*-hydroxytrimethylene) piperazine-2,5-dione (26): Obtained from diketopiperazine (**24**) (222 mg, 0.5 mmol) according to the general procedure for deprotection of benzyl ether in diketopiperazines, extrapolating (3.3-fold) the general amounts. The reaction mixture was purified by rotatory chromatography (CH_2Cl_2/MeOH 98:2), yielding compound **25** (77 mg, 43%) and compound **26** (39 mg, 26%).

Compound 25: Syrup; $[\alpha]_D$ = −83 (c 0.20, $CHCl_3$). ^{1}H NMR (500 MHz, CD_3OD): δ_H 1.23 (dd, *J* = 6.9, 7.3 Hz, 3H), 1.47 (m, 1H), 1.62 (m, 1H), 1.99–2.09 (m, 2H), 2.05 (s, 3H), 2.16 (m, 1H), 2.29–2.44 (m, 3H), 3.40 (d, *J* = 12.9 Hz, 1H), 3.82 (dd, *J* = 4.7, 12.9 Hz, 1H), 4.11 (ddd, *J* = 6.9, 6.9, 7.0 Hz, 2H), 4.41 (m, 1H), 4.46 (m, 1H), 4.52 (dd, *J* = 6.0, 11.4 Hz, 1H), 5.39 (d, *J* = 10.4 Hz, 1H), 5.70 (d, *J* = 10.7 Hz, 1H). ^{13}C NMR (125.7 MHz, CD_3OD): δ_C 14.5 (CH_3), 20.4 (CH_3), 20.6 (CH_2), 28.9 (CH_2), 34.5 (CH_2), 39.4 (CH_2), 55.5 (CH_2), 58.4 (CH), 60.3 (CH), 61.6 (CH), 68.5 (CH_2), 68.6 (CH_2), 166.3 (C), 171.6 (C), 171.9 (C), 174.9 (C). IR ($CHCl_3$) ν_{max}= 3456, 1732, 1672, 1575, 1447 cm^{-1}. HRMS (ESI-TOF) calcd for $C_{16}H_{24}N_2O_7Na$ (M^+ + Na) 379.1481, found 379.1487. Anal. Calcd for $C_{16}H_{24}N_2O_7$: C, 53.93; H, 6.79; N, 7.86. Found: C, 53.92; H, 6.88; N, 7.76.

Compound 26: Syrup; $[\alpha]_D$ = −58 (c 0.20, $CHCl_3$). ^{1}H NMR (500 MHz, CD_3OD): δ_H 1.23 (dd, *J* = 6.9, 7.3 Hz, 3H), 1.32 (m, 1H), 1.45 (m, 1H), 1.95 (ddd, *J*

= 4.7, 12.3, 12.6 Hz, 1H), 2.03 (m, 1H), 2.16 (m, 1H), 2.29–2.42 (m, 3H), 2.99 (s, 3H), 3.33 (d, *J* = 12.9 Hz, 1H), 3.91 (dd, *J* = 5.4, 13.3 Hz, 1H), 4.10 (ddd, *J* = 6.9, 7.0, 7.3 Hz, 2H), 4.24 (m, 1H), 4.43–4.49 (m, 2H). ^{13}C NMR (125.7 MHz, CD_3OD): δ_C 14.5 (CH_3), 19.7 (CH_2), 29.7 (CH_2), 31.5 (CH_3), 34.3 (CH_2), 40.4 (CH_2), 55.7 (CH_2), 58.1 (CH), 61.5 (CH_2), 62.3 (CH), 68.3 (CH), 166.1 (C), 169.6 (C), 174.8 (C). IR ($CHCl_3$) ν_{max}= 3450, 1727, 1660, 1449 cm^{-1}. HRMS (ESI-TOF) calcd for $C_{14}H_{22}N_2O_5Na$ (M^+ + Na) 321.1426, found 321.1425. Anal. Calcd for $C_{14}H_{22}N_2O_5$: C, 56.36; H, 7.43; N, 9.39. Found: C, 56.11; H, 7.58; N, 9.20.

(3*S*,6*S*)-1,4-diacetoxymethyl-6-(4-ethoxy-4-oxo-2-butenyl)-3,4-(2-morpholinoethyl)piperazine-2,5-dione (27): Obtained from diketopiperazine (**25**) (71 mg, 0.2 mmol) according to the general procedure for the scission of 4-hydroxyproline derivatives and reductive amination with secondary amines, using morpholine (21 µL, 0.24 mmol) as the secondary amine. The reaction mixture was purified by rotatory chromatography (CH_2Cl_2/MeOH 99:1), yielding compound **27** (49 mg, 50%), a syrup; $[\alpha]_D$ = −31 (c 0.34, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$): δ_H 1.23 (dd, *J* = 7.0, 7.3 Hz, 3H), 1.74 (m, 1H), 1.83 (m, 1H), 1.89 (m, 1H), 1.99 (m, 1H), 2.05–2.18 (m, 2H), 2.08 (s, 6H), 2.35 (ddd, *J* = 2.5, 6.9, 6.9 Hz, 2H), 2.46–2.70 (m, 6H), 3.68–3.78 (m, 4H), 4.11 (ddd, *J* = 7.0, 7.3, 7.3 Hz, 2H), 4.14 (dd, *J* = 5.0, 8.5 Hz, 1H), 4.32 (dd, *J* = 6.6, 6.6 Hz, 1H), 5.31 (d, *J* = 10.4 Hz, 1H), 5.39 (d, *J* = 10.0 Hz, 1H), 5.52 (d, *J* = 10.4 Hz, 2H). ^{13}C NMR (125.7 MHz, $CDCl_3$): δ_C 14.2 (CH_3), 20.76 (CH_3), 20.79 (CH_2), 21.2 (CH_3), 31.4 (CH_2), 33.2 (CH_2), 33.5 (CH_2), 53.2 (2 × CH_2), 54.3 (CH_2), 57.7 (CH), 60.0 (CH), 60.5 (CH_2), 66.4 (2 × CH_2), 68.3 (CH_2), 68.6 (CH_2), 166.7 (C), 167.2 (C), 170.4 (C), 170.5 (C), 172.7 (C). IR ($CHCl_3$) ν_{max}= 1713, 1418, 1364 cm^{-1}. HRMS (ESI-TOF) calcd for $C_{22}H_{35}N_3O_9Na$ (M^+ + Na) 508.2271, found 508.2272. Anal. Calcd for $C_{22}H_{35}N_3O_9$: C, 54.42; H, 7.27; N, 8.65. Found: C, 54.42; H, 7.15; N, 8.41.

(3*S*,6*S*)-1-methyl-4-acetoxymethyl-6-(4-ethoxy-4-oxo-2-butenyl)-3,4-(2-morpholinoethyl)-piperazine-2,5-dione (28): Obtained from diketopirazine **26** (30 mg, 0.1 mmol) according to the general procedure for the scission of 4-hydroxyproline derivatives and reductive amination with secondary amines, using morpholine as the secondary amine (reducing the general amounts by half). The reaction mixture was purified by rotatory chromatography (CH_2Cl_2/MeOH 99:1), yielding compound **28** (22 mg, 52%), a syrup; $[\alpha]_D$ = −19 (c 0.40, $CHCl_3$). ^{1}H NMR (500 MHz, CD_3OD): δ_H 1.23 (dd, *J* = 6.9, 7.3 Hz, 3H), 1.69 (m, 1H), 1.75–1.89 (m, 2H), 1.96–2.16 (m, 3H), 2.06 (s, 3H), 2.39 (ddd, *J* = 6.9, 7.3, 7.3 Hz, 2H), 2.45–2.58 (m, 6H), 2.97 (s, 3H), 3.65–3.74 (m, 4H), 4.02 (dd, *J* = 4.7, 8.2 Hz, 1H), 4.11 (ddd, *J* = 7.0, 7.3, 7.3 Hz, 2H), 4.29 (dd, *J* = 5.7, 7.3 Hz, 1H), 5.45 (d, *J* = 10.4 Hz, 1H), 5.49 (d, *J* = 10.1 Hz, 1H). ^{13}C NMR (125.7 MHz, CD_3OD): δ_C 14.6 (CH_3), 20.7 (CH_3), 22.4 (CH_2), 32.5 (CH_2), 33.2 (CH_3), 33.4 (CH_2), 34.2 (CH_2), 54.6 (2 × CH_2), 55.7 (CH_2), 59.6 (CH), 61.5 (CH_2), 63.3 (CH), 67.7 (2 × CH_2), 70.4 (CH_2), 168.2 (C), 169.1 (C), 172.1 (C), 174.7 (C). IR ($CHCl_3$) ν_{max}= 1733, 1711, 1670, 1458 cm^{-1}. HRMS (ESI-TOF) calcd for $C_{20}H_{33}N_3O_7Na$ (M^+ + Na) 450.2216, found 450.2213. Anal. Calcd for $C_{20}H_{33}N_3O_7$: C, 56.19; H, 7.78; N, 9.83. Found: C, 56.29; H, 7.82; N, 9.52.

(3*S*,6*S*)-1,4-di(3-butenyl)-6-(4-ethoxy-4-oxo-2-butenyl)-3,4-(2-morpholinoethyl)-piperazine-2,5-dione (29): A solution of the diketopiperazine **27** (48 mg, 0.1 mmol) in dry MeCN (2 mL) was cooled to 0 °C and treated with allyltrimethylsilane (159 µL, 1.0 mmol) and TMS-OTf (72 µL, 0.4 mmol). The reaction mixture was stirred for 16 h, and then was poured into saturated aqueous $NaHCO_3$ and extracted with EtOAc. The organic layer was dried and the solvent was removed as usual. The residue was purified by rotatory chromatography on silica gel (EtOAc), yielding compound (**29**) (30 mg, 67%), as a syrup; $[\alpha]_D$ = −10 (c 0.27, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$): δ_H 1.23 (dd, *J* = 7.1, 7.3 Hz, 3H), 1.68 (m, 1H), 1.75–1.87 (m, 2H), 1.91–2.05 (m, 2H), 2.10 (m, 1H), 2.27–2.34 (m, 2H), 2.36–2.42 (m, 4H), 2.66 (br b, 4H), 2.70 (dd, *J* = 7.3, 7.3 Hz, 2H), 2.99–3.08 (m, 2H), 3.72 (dd, *J* = 4.6, 4.6 Hz, 4H), 3.84–3.93 (m, 2H), 3.94 (dd, *J* = 4.7, 8.4 Hz, 1H), 4.07 (dd, *J* = 4.9, 8.4 Hz, 1H), 4.09 (d, *J* = 7.1 Hz, 1H), 4.12 (d, *J* = 7.1 Hz, 1H), 5.01–5.09 (m, 4H), 5.74–5.83 (m, 2H). ^{13}C NMR (125.7 MHz, $CDCl_3$): δ_C 14.5 (CH_3), 22.5 (CH_2), 31.7 (CH_2), 32.5 (CH_2), 32.6 (CH_2), 34.0 (CH_2), 34.2 (CH_2), 45.5 (CH_2), 45.7 (CH_2), 54.4 (2 × CH_2), 56.0 (CH_2), 59.5 (CH), 61.5 (CH_2), 61.7 (CH), 67.3 (2 × CH_2), 117.8 (2 × CH_2), 136.0 (2 ×CH), 168.2 (C), 168.4 (C), 174.8 (C). IR ($CHCl_3$) ν_{max}= 1725, 1655, 1472 cm^{-1}. HRMS (ESI-TOF) calcd for $C_{24}H_{39}N_3O_5Na$ (M^+ + Na) 472.2787, found 472.2794. Anal. Calcd for $C_{24}H_{39}N_3O_5$: C, 64.12; H, 8.74; N, 9.35. Found: C, 64.49; H, 8.99; N, 9.70.

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Synthesis of Diketopiperazine Scaffolds with tailored *N*- and α-chains by Selective Modification of Customizable Units

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